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RESEARCH ARTICLE

Optimizing multivariate behavioural syndrome models in locusts using automated video tracking.

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RESEARCH ARTICLE

Optimizing multivariate behavioural syndrome models in locusts using automated video tracking.

HIGHLIGHTS

1. Locusts exhibit a behavioural syndrome known as phase polyphenism
2. Automated video tracking allows optimization of multivariate behavioural models
3. Model optimization unveils novel behavioural relationships within the syndrome

ABSTRACT

Locusts exhibit a behavioural syndrome known as ‘behavioural phase polyphenism’, in which a number of behavioural traits change markedly in response to local population density. 'Solitarious' phase individuals, which are typical of low density populations, change within hours from being relatively sedentary and repelled by other locusts to actively congregating with conspecifics and becoming more active (the 'gregarious' phase). In wild populations, this behavioural plasticity can lead to the emergence of mass marching bands of nymphs and winged adult swarms. Much of our understanding of behavioural phase transition comes from laboratory experiments, which routinely employ an arena-based assay to measure a suite of behavioural variables encompassing aspects of activity, movement pattern and responses towards a stimulus group of other locusts. Individuals are then quantitatively phenotyped along a linear scale from solitarious to gregarious, by entering their returned measurements for several behavioural characters into a logistic regression model. Recently, automated video tracking has enabled multiple experimenters to use a single

behavioural model, rather than each having to construct their own. Here, we have taken advantage of another powerful feature of automated tracking systems: the opportunity to use stored data to conduct a rigorous optimization process, which both ensures that the derived statistical model encapsulates the multidimensional nature of locust behavioural phase to best effect, and also provides a new understanding of the relationship between different behaviours.

KEY WORDS

Australian plague locust, behavioural modelling, behavioural syndrome, *Chortoicetes terminifera*, density-dependent, locust phase polyphenism, logistic regression, phenotypic plasticity

INTRODUCTION

Individual variation in animal behaviour can often be attributed to what has become known as a 'behavioural syndrome', in which a suite of multiple behavioural traits consistently co-vary across time, or across a range of contexts (Sih et al. 2004; Bell 2007; Dingemanse & Wolf 2010). Despite an increasing interest in the evolution and ecological importance of behavioural syndromes, the methods used to score the behavioural state of individuals typically measure a single behaviour as a surrogate for the entire syndrome. For example, 'boldness' in fish is frequently assessed by introducing a novel item close to the individual, and monitoring its subsequent spatial response; animals that tend to remain within 5 cm of the novel object are classified as bold, those beyond 10 cm as shy, and animals in between as intermediate (e.g. Wilson et al. 1993; Frost et al. 2007). One of the few study systems in which efforts have

been made to combine multiple traits comprising a behavioural syndrome into a single predictive measurement is research into locust behaviour (Roessingh et al. 1993; Pener & Simpson 2009).

Locusts exhibit an extreme example of phenotypic plasticity known as phase polyphenism, in which a single genotype can give rise to a broad spectrum of adaptive phenotypes in response to local environmental cues. The trait for which locusts are best known is their propensity to form migratory bands and swarms, as a result of a density-dependent behavioural transition at the level of the individual (Uvarov 1966; Simpson & Sword 2009; Pener & Simpson 2009). With increasing population density, locusts will transition from a solitarious phase exhibiting low levels of activity and the avoidance of conspecifics towards a more active gregarious phase, that displays pronounced aggregating behaviour that can lead to economically important outbreaks in agricultural systems worldwide (Sword et al. 2010). In crowded conditions, this behavioural shift begins to occur after only a few hours, with other phase-related traits (including variations in colour, morphology and physiology) following in successive nymphal stadia and, in some cases, over successive generations (Pener & Simpson 2009; Tanaka & Maeno 2010).

The underlying physiological processes that govern locust phase transition are being steadily unraveled, whether it be the mechanisms by which locusts are stimulated by one another (Simpson et al. 2001; Rogers et al. 2003; Cullen et al. 2010; Maeno et al. 2011), associated changes in neuronal function (Rogers et al. 2004; Anstey et al. 2009; Ma et al. 2011; Ott et al. 2012) or shifts in gene expression (Guo et al. 2011). In all cases, assigning mechanistic function depended critically upon the ability to measure fine-grained changes in behavioural phase state. Peggy Ellis (e.g. 1953, 1959, 1963a,b) pioneered the assaying of behavioural state in locusts (review of

various methods in Pener & Simpson 2009) and her inspiration gave rise to what has become the most widely employed methodology for doing this in recent years, the assay devised by Roessingh et al. (1993) for the desert locust, *Schistocerca gregaria*. This assay has been used extensively to quantify the time-course of behavioural phase change and to explore its underlying mechanisms in *S. gregaria* and several other species of acridid grasshopper (see Supplementary Table 1).

The first step in the Roessingh et al. (1993) system is to record the behaviour of a large number (typically 50-150 per category) of 'model' insects of known crowding history (solitary-reared and crowd-reared) when placed singly into a standard arena (Gray et al. 2009; see Materials and Methods for more details). During the assay, individual locusts are monitored for 5 – 10 min, with the observer recording values for a suite of behaviours that can be broadly divided into two categories; those that relate to activity (e.g. distance moved, turning, grooming, climbing), and those that account for the individual's proximity to a 'stimulus group' of at least 20 conspecifics placed behind a clear partition at one end of the arena. Gregarious phase locusts typically record a high overall level of activity and are attracted to the group, whereas solitarious individuals move much less and are actively repelled by other insects (Simpson et al. 1999). Returned values for the broad suite of behavioural variables are then used to build a binary logistic regression model, which selects the specific combination and weighting of variables that most strongly separates the locusts by phase, before weighting and collapsing this selection into a single quantitative descriptor. This is typically designated as either *P*(greg), the probability that an individual belongs to the gregarious phase, or *P*(sol), its converse for solitarious phase locusts.

With a behavioural model established, experimental locusts that have been subjected to some experimental perturbation (different periods of crowding or isolation, sensory stimulation, drug treatment, gene expression manipulation, etc.) can then be assayed in the same arena, with returned values for the selected behavioural variables being entered into the logistic regression model. This yields a score of *P*(greg) along a linear scale between 0, where the locust is behaviourally indistinguishable from the solitary-reared model population, and 1, where the insect is indistinguishable from the gregarious subset. Intermediate values denote a transitional behavioural phase state.

Subjective differences in interpretation of behaviours by human observers are an important constraint on comparisons of any type of behavioural data. In studies of locust behavioural phase change, these subtle but important nuances in observational opinion have necessitated that the same user performs all the behavioural observations for a given experiment to ensure that the behavioural phenotype of each test locust is consistently defined. Over recent years, the issue of user-specificity has been largely overcome by the gradual replacement of manual observation with automated video tracking software (Gray et al. 2009; Cullen et al. 2010; Guo et al. 2011). The greatest cost associated with automating the assay system in this way is the loss of detection of some subtle body movements such as grooming and shifts in posture (Pener & Simpson 2009). Nevertheless, automated systems are able to record both gross activity-related variables (e.g. distance moved, climbing, and variables associated with turning) and an insect's position in the behavioural arena with a high degree of accuracy. Furthermore, for smaller study insects (younger age classes, or smaller species) the automated assay can also be twice as time efficient as manual observation since two arenas (and potentially more) can be filmed and assayed at once.

Given the demonstrated utility of automated video tracking for quantitative assessments of multivariate behaviours, an important and previously unaddressed advantage is that it becomes possible to optimize the assay system to ensure that behaviours are recorded and analyzed in a biologically meaningful way, while maximizing the efficiency of the assay for streamlined behavioural phenotyping. Such an optimization procedure would make a valuable contribution not only to locust research, but also to other researchers considering taking advantage of the power of such an approach to deal with multiple behavioural variables in behavioural assays. Here, we employ the Australian plague locust, *Chortoicetes terminifera* (Walker), to optimize a range of individual behavioural variables recorded by an automated video tracking system. We then evaluate the use of these variables in the process of constructing a robust behavioural logistic regression model that accounts for the multi-dimensional nature of locust behavioural phase.

MATERIALS AND METHODS

Insects

Australian plague locusts were reared at the University of Sydney, according to established protocols (Gray et al. 2009). Locusts were collected from wild populations in Western Australia and New South Wales, and reared under constant crowded conditions for multiple generations to maintain a gregarious locust stock. Solitarious locusts were obtained by removing nymphs from this gregarious culture within 2 days of hatching, and rearing them in visual, olfactory and physical isolation in a separate controlled-temperature insectary. This period of isolation is sufficient to

produce fully solitarious behavioural characteristics in this species (Gray et al. 2009; Cullen et al. 2010), with no risk of genetic differentiation between the two phases, as occurs when solitarious cultures are run in isolation over multiple generations (Berthier et al. 2010).

Experimental insects were assayed for behaviour during their final nymphal stadium, 2 – 3 days after moulting. In total, 128 gregarious and 123 solitarious individuals were used, with each animal being assayed only once.

Behavioural phase assay

The behavioural arena and assay were originally devised for the desert locust, *S. gregaria*, and have been described in detail elsewhere (Roessingh et al. 1993; Simpson et al. 1999). The Australian plague locust is smaller in size than *S. gregaria*, and can thus be assayed in a smaller behavioural arena. When coupled with an automated tracking system, this enabled the accurate assaying of two insects at once in separate side-by-side arenas (described in detail in Gray et al. 2009). Briefly, the assay setup consisted of two rectangular arenas, each measuring 23 cm long × 15 cm wide × 10 cm high. The long walls of each arena were built from white Perspex, while the end walls were constructed of clear, perforated Perspex, behind only one of which was placed a perforated clear container containing 20 final-instar nymphs from the gregarious culture: the 'stimulus group'. An empty but otherwise identical container was positioned behind the other end wall, with all four containers concurrently backlit by a 25W fluorescent tube at each end. The lid of each arena was made from clear Perspex, while the floor was covered in fresh white paper for each experimental day. Locusts were introduced into each arena through a 2 cm hole in the centre of the arena

floor using a 15 mL syringe, modified to have a 2 cm wide exit and screw cap, in which they were first allowed to settle for 5 min. The behaviour of each insect was recorded in real time for 8 min by a colour CCTV camera, and converted into frame-by-frame data by a PC installed with a Piccolo video frame-grabber. During each assay, raw activity and position-related data were acquired for all locusts using Ethovision® 3.1 (Noldus et al. 2002; Noldus Information Technology 2005).

Behavioural analysis and statistics

Once all behavioural tracks had been gathered, the first step was to optimize the 'Minimum Distance Moved' (MDM) input filter, which removes inherent noise from the tracks so that Ethovision retains only true body movement of the locusts for analysis. This was particularly important with animals as small as *C. terminifera* (1 – 2 cm long as final-instar nymphs), because the pixilated nature of the CCTV output would frequently not allow a test insect's centre of mass (COM) to be precisely determined. This resulted in the tracking 'crosshair' scanning back and forth along the length of the insect while it was stationary, and incorrectly recording this artifact as displacement; a phenomenon known as 'body wobble' (Hen et al. 2004; Noldus Information Technology 2005). Fortunately, properly optimized input filters remedy this problem very effectively. The MDM filter creates a lower threshold of movement per second, below which any change in a test insect's COM is not recorded as true movement. We performed 11 iterations of MDM (every 1 mm from 0 – 10 mm) to find the filter that yielded the most acceptable level of assay noise. For each iteration, mean values for each of four activity-related variables were returned for all 251 locusts, and graphed with PASW Statistics 18.0 (SPSS Inc., Chicago, IL) to ensure

that the filter setting did not affect the returned values disproportionately by phase. The four activity-related variables assessed were: ‘total distance moved’, ‘mean velocity’, ‘mean angular velocity’ (a measure of turning speed), and ‘mean meander’ (a measure of path tortuosity). Body wobble did not affect the values returned for position-related variables, since the false displacements over the length of the test locust would invariably average out at the coordinate of the true COM over the period in which the insect was resting.

Having established the optimum MDM filter setting, we investigated the differences between solitarious and gregarious locusts for a range of behaviours related to overall activity, position relative to the stimulus group of conspecifics, and climbing of the arena walls. Where appropriate, behaviours were also considered across the duration of the assay to identify variables that differed over time. These analyses were all performed in PASW Statistics 18.0; a full list of variables is given in Table 1.

Behavioural variables that informatively differed between solitarious and gregarious locusts across the 8-min assay duration were entered into a forward step-wise conditional logistic regression analysis in PASW Statistics 18.0, to select and collapse the most informative variables into the single measure of behavioural phase, *P*(greg). Significance values for entry and removal of a variable from the model were set to 0.05 and 0.10, respectively (the default settings). The fit of the model was formally assessed using the Hosmer-Lemeshow goodness-of-fit test, in which a robust model returns a non-significant *P*–value for a χ^2-related test statistic (Hosmer & Lemeshow 2000). Additional models were built using the values returned for the same behaviours after the first 2, 4 and 6 min of the assay, to assess the impact of trial

duration on the behavioural model. A further model was also built using all unrefined variables, to validate the variable optimization process.

RESULTS

Input filter determination

The effects of 11 MDM input filter settings on four activity-related variables are summarized in Fig 1. In the first instance, the most important variable for consideration was total distance moved, from which values for all other activity-based variables were at least partly dependent. As Fig. 1a demonstrates, the mean value for total distance moved sharply decreased to a point of inflection at a MDM setting of 2 – 3 mm, supporting a MDM filter within this range. Lower MDM settings resulted in a notable increase in total distance moved, which can be attributed to noise resulting from body wobble (Hen et al. 2004; Noldus Information Technology 2005). Conversely, increasing the MDM value beyond 3 mm caused a less severe but continual decrease in total distance moved, due to the gradual loss of genuine displacement information. A similar pattern was also apparent for 'mean angular velocity' (Fig. 1c), with a point of inflection at a MDM of 2 – 3 mm. Although this stringency returned a reduced disparity between the two phases, it remained a highly informative variable.

'Mean velocity' exhibited a notably different pattern in response to altered filter settings (Fig. 1b). The lowest mean velocities were apparent where MDM was set to 0 mm, which resulted from Ethovision calculating mean velocity by dividing the total distance moved by the time spent moving, and not the overall time of the

assay. At a MDM of 0 mm, Ethovision attributed each locust not only an inflated value for total distance moved due to body wobble noise, but also a disproportionately increased value for movement time even though the insect was not actually moving, returning an unrealistically low velocity per locust. Conversely, increasing MDM beyond 3 mm had a disproportionate effect on mean velocity by phase, with the returned values for gregarious insects remaining constant while solitarious values gradually reduced. The MDM filter thus appeared to remove informative displacement data from solitarious tracks above a stringency of 3 mm, whilst introducing an unacceptable level of noise to all tracks when set below 2 mm. This lent further weight to our observations based on total distance moved, that a MDM between 2 – 3 mm was the optimal setting for this filter.

'Mean meander' displayed an unexpected pattern in response to altering the input filters; a MDM of only 1 mm reduced the meander markedly (Fig. 1d). The point of inflection along the MDM axis occurred earlier than for the other activity-related variables, at around 1 – 2 mm, while a MDM of 3 mm substantially reduced the returned meander values for both phases. As the only behavioural variable that did not support the consensus MDM filter setting of 3 mm, meander was subsequently dropped from further consideration in the model-building process.

Activity-related behavioural variables

Having applied the MDM filter of 3 mm to all 251 tracks, the differences between solitarious and gregarious locusts for the remaining three activity-related variables could be assessed across the 8-min duration of the assay (Fig. 2a-c). Furthermore, by rank-normalizing the returned data for each variable (Simpson et al.

1999), we were able to perform simple univariate ANOVAs to determine the effects, if any, of both locust phase and portion of the assay time on each behaviour. Gregarious locusts moved, on average, 13.9 (SD = 3.4, $N = 8$) times more than solitarious locusts per assay minute (ANOVA: $F_{1, 2352} = 1396.5$, $P < 0.001$), and this did not differ significantly across the duration of the assay (ANOVA: $F_{7, 2352} = 1.085$, $P = 0.370$; Fig. 2a). Gregarious insects were also faster than their solitarious counterparts (ANOVA: $F_{1, 2352} = 638.53$, $P < 0.001$), although locusts of both phases slowed down significantly as the assay progressed (ANOVA: $F_{7, 2352} = 2.233$, $P = 0.029$; Fig. 2b). Normal scores for total distance moved and mean velocity correlated positively, but the relationship was weaker in the solitarious phase insects, in which two distinct subsets were apparent (Fig. 2d). Since this disparity potentially contributed some informative phase-related information (discussed later), we included both 'total distance moved' and 'mean velocity' in the list of variables for consideration in the logistic regression model.

Mean angular velocity was significantly higher in solitarious locusts (ANOVA: $F_{1, 2352} = 382.62$, $P < 0.001$), and did not alter significantly across the assay duration (ANOVA: $F_{7, 2352} = 0.674$, $P = 0.694$). As demonstrated by the 95% CIs in Fig. 2c, there was considerable overlap between the returned values for the two phases, with the greatest variability apparent in the solitarious subset. This disproportionate distribution was also noticeable during input filter assessment (Fig. 1c). Further investigation of mean angular velocity across the entire assay duration (Fig. 2e) highlighted the underlying suite of behaviours responsible for this pattern. The distribution of mean angular velocities among the gregarious subset approached normality, with most insects displaying a degree of turning during their movement around the arena. By contrast, the solitarious subset was far from Gaussian in

distribution. A large number of these insects did not move at all after initial entry to the arena, and thus recorded an angular velocity of 0 degrees/s, while others had angular velocities above 150 degrees/s. Upon inspection of the corresponding individual tracks for these insects, it was clear that many solitarious locusts spent the majority of the assay performing small movements on or near the arena walls, without any substantial associated change in location. These movements included a large amount of slow turning on the spot in some instances, as well as a number of small subtle movements. Not all of these were turning movements, but were nonetheless recorded by Ethovision. It was previously thought that Ethovision could not detect subtle activities (Gray et al., 2009; Pener & Simpson, 2009) but, given the clear phase-dependent pattern retained by our filter settings, we opted to rename this variable 'turning and small body movements', and include it in the list of variables for consideration by the logistic regression model.

As all three activity-related behaviours varied significantly and consistently by phase across each minute of the assay, we collapsed each of them into a single value for the entire 8-min duration, leaving 'total distance moved', 'mean velocity' and 'turning and small body movements' for consideration in the final logistic regression model (described later).

Position-related behavioural variables

To assess the strength of attraction to the stimulus group within the confines of the behavioural assay, we first divided the floor of the arena into 23 sections, each of 1 cm width, parallel to the end walls of the arena. The stimulus and non-stimulus walls were treated as separate sections, and a mean duration within each zone was

then recorded for every 60 s segment of the total assay duration. A clear difference can be noted between solitarious and gregarious locusts (Fig. 3).

During the first minute of the assay, both solitarious and gregarious locusts spent time around the centre of the arena, the region in which they entered. Over increasing minutes, solitarious locusts gradually spent more time at the non-stimulus end of the arena, and typically occupied either the non-stimulus wall or the immediately adjacent area by the time the assay had finished. Solitarious locusts spent very little time towards the stimulus end of the arena, with not a single individual spending any amount of time climbing the stimulus wall. This strong repulsion was contrasted by the behaviour of the gregarious locusts, of which some spent time climbing the stimulus wall. While this renders this variable a perfect predictor of gregarious phase, little more than half of the gregarious locusts did this (54.7%; raw data, not shown). Many gregarious insects approached the stimulus wall, but did not actually climb it. Interestingly, gregarious insects tended to spend more time than solitarious insects climbing the non-stimulus wall in the first half of the assay, with the trend shifting to solitarious insects for the second half as gregarious insects eventually settled closer to the stimulus group, and solitarious insects away from the group.

Since the times spent on the two end walls were not universally informative on their own, we opted to collapse these data into that of the respective end zones, leaving three variables that best represented the overall difference in spatial distribution observed between the two phases. Shown in Supplementary Fig. 1a, these were; (i) time spent in the stimulus third of the arena, including the stimulus wall (sections 0 – 7 from Fig. 3), (ii) time spent in the centre third of the arena (sections 8 – 16 from Fig. 3), and (iii) time spent in the non-stimulus third of the arena, including

the non-stimulus wall (sections 17 – 24 from Fig. 3). Given that all insects began the assay in the centre third of the arena, and that the difference between solitarious and gregarious locusts for this measure was only slight, it was not included for consideration by the final behavioural model (see Table 1).

Despite the clear phase-related differences in position apparent from Supplementary Fig. 1a, it is again important to note that gregarious insects spent almost exactly as much time at the non-stimulus end of the arena as they did next to the group of conspecifics. While this was clearly due to their increased activity relative to solitarious locusts, the net result was that the general tendency for gregarious locusts to move towards the group over time (apparent in Fig. 3) was not accounted for once these data were compressed into arena thirds. To overcome this, we also recorded the distance from the stimulus group at the end of the 8-min trial for each locust. This variable has been the single most predictive position-related variable in the majority of previous studies, across at least three species (e.g. Roessingh et al. 1993; Sword 2003; Chapuis et al. 2008), and is generally referred to as 'X–distance', the label we have given it here (shown in Supplementary Fig. 1b).

Climbing

For the purpose of our Ethovision-based analyses, a locust was deemed to be climbing at any point that its COM was recorded on one of the four arena walls. Given the clear disparity in phase-related movement, we aimed to show definitively whether climbing variables are discrete predictors of phase, or else result from a differential propensity to encounter the arena walls. We investigated this possibility by analyzing the subset of locusts that spent time climbing (163 individuals). Rank-

normalized values for five variables were used; (i) distance moved, (ii) the cumulative time spent climbing both the stimulus and non-stimulus walls (see previous section, and Fig. 3), (iii) total time spent climbing all four arena walls (a position-biased variable), (iv) frequency of individual climbing events throughout the track duration (an activity-biased variable), and (v) mean duration of each climbing event (a potential measure of both activity and position).

Total climbing time correlated strongly with the cumulative time spent on both end walls, for both phases (Supplementary Fig 2a). Only a small number of insects appeared to spend time climbing the two long walls of the arena, and this was probably due to the fact that only the end walls were backlit. Values for time spent on the stimulus and non-stimulus walls were previously collapsed into their adjacent thirds of the arena floor in the last section which considered insect position, and were thus accounted for already; total climbing time contributed little additional behavioural information. Furthermore, 'stimulus third time' and 'non-stimulus third time' accounted for the presence of the stimulus group of conspecifics, whereas climbing time was only a generic measurement.

Climbing frequency correlated positively with distance moved for gregarious locusts (Supplementary Fig. 2b), suggesting that this measure of climbing activity was simply a function of overall movement (and thus, increased wall encounters) for these insects. The scatter was somewhat different for solitarious phase locusts, and serves to highlight two noted characteristics of these animals. Those represented below the fitted line moved a short distance before perching on the non-stimulus wall for the remainder of the assay, obtaining a climbing frequency of only 1 or 2. Those represented above the fitted line moved a similarly short distance, but mounted the arena walls a higher number of times as they made a number of turns and small

movements at the floor-wall interface. Since these two solitarious traits were already accounted for by 'total distance moved' and 'turning and small body movements', climbing frequency did not contribute any additional behavioural insights.

Mean climb duration correlated strongly with total climbing time (Fig. 4a), while there was no correlation with distance moved (data not shown). Solitarious insects exhibited a higher mean climb duration than did their gregarious counterparts, despite the fact that the gregarious insects spent more time climbing overall (Fig. 4b). Since mean climb duration potentially contributed some novel behavioural insight (discussed later), it was the only climbing variable carried forward for consideration in the final behavioural model.

Building and testing the logistic regression model

Having assessed the utility of all potential behavioural variables, a total of seven were deemed to be of sufficient biological relevance in discriminating locust phase difference to be included in the model-building process (see Table 1). Variables were entered into a forward step-wise conditional logistic regression analysis to select and collapse the most informative variables into a single linear predictor of gregariousness, *P*(greg)(Hosmer & Lemeshow 2000; Pener & Simpson 2009). Models were built using returned values for the entire 8-min assay duration, and for values recorded for the first 2, 4 and 6 min of the same dataset, to assess the importance of assay duration. In addition, a further logistic regression was performed to assess the necessity of the variable refinement and selection process detailed in previous sections. Using values returned for the full 8-min assay duration, we entered the entire, unrefined list of 13 variables for consideration by the logistic regression model

(listed in Table 1). For all models, activity-related variables were still subject to the optimum 3 mm MDM filter.

The 8-min model built from the refined variable subset correctly classified 92.4% of locusts, retaining total distance moved, turning and small body movements, and total time spent in the non-stimulus third of the arena. Models built from the 2, 4 and 6-min datasets were not as efficient predictors of phase as the model using the full 8-min dataset (Table 2b-d). The 2-min model retained total distance moved and mean velocity, and only assigned the correct phase to 78.5% of the 251 locusts. After 4 and 6 min, the models also retained the positional variable X–distance alongside total distance moved and mean velocity, and had an increased phase prediction accuracy (82.5% and 83.7% of locusts correctly assigned, respectively). The 8-min model built using the unrefined list of variables (Table 2e) correctly classified 91.7% of the locusts, and retained total distance moved, meander, turning and small body movements, and centre third time. Across the five models constructed, only the 8-min model, built using the refined list of variables, achieved a non-significant (thus acceptable) value for the Hosmer-Lemeshow goodness-of-fit test ($P = 0.945$, d.f. = 8) (Table 2a). The other four models scored poorly in this assessment, with significant values for the calculated test statistics.

To further investigate the interaction between variables, we set both 'turning and small body movements' and 'non-stimulus third time' to the highest values found in the solitarious subset (150 degrees/s and 480 s, respectively) to determine the 'distance moved' value that would nevertheless return a *P*(greg) value above 0.5 (i.e. closer to a fully gregarious score of 1.0 than a fully solitarious one of 0.0). At the opposite extreme, the same two variables were set to the lowest values found within the gregarious dataset (1.7 degrees/s and 0 s), before again determining the value for

distance moved that returned a *P*(greg) value higher than 0.5. These distances were found to be 755 mm and 105 mm, respectively, and it is thus within this range of distance moved that values for the other two variables were able to exert a meaningful influence on the returned score of *P*(greg).

To demonstrate this principal, distance moved was then set to the mean value of the two distances determined above (430 mm), to allow the interactive effect of the other two variables to be elucidated (Fig. 5). As expected from the Wald significance values (Table 2a) 'turning and small body movements' had more of an effect on *P*(greg) than did 'non-stimulus third time'; at a distance moved of 430 mm, any value for 'turning and small body movements' below 54.1 degrees/s returned a *P*(greg) value that was closer to fully gregarious, while values above 97.7 degrees/s categorized a locust as being closer to fully solitarious.

DISCUSSION

The locust behavioural assay system developed by Roessingh et al. (1993) has provided the foundation for much research into both the mechanisms underlying density-dependent behavioural polyphenism and its implications for collective behaviour and mass migration. The multivariate logistic regression model derived from the assay calculates a single predictor of locust behavioural phase state, *P*(greg), which encapsulates multiple dimensions within this behavioural sydrome. Here we have used automated video tracking both to optimize assay settings and to aid in the selection of behavioural variables for inclusion in models.

Input filter determination

The accurate behavioural characterization of the solitarious and gregarious phases forms the foundation of the Roessingh et al. (1993) locust assay system. It is from these two extremes of behaviour that the logistic regression model is built, with all subsequent test insects placed on a vector between the two multidimensional definitions. We have shown here the potential for input filters to contribute towards phase-dependent recording biases unless appropriately set.

Mean values for 'total distance moved', 'mean velocity' and 'turning and small body movements' (formerly mean angular velocity) all supported a Minimum Distance Moved filter setting of 3 mm, with lower MDM settings introducing an unacceptable level of noise and higher settings gradually removing genuine movement data. Optimization of this filter came at the expense of the meander variable, which we were forced to omit from further consideration due to its incompatibility with this filter setting. Encouragingly, a MDM filter of 3 mm mirrors previous work employing automated tracking in analyses of *C. terminifera*, where it was set to 3.3 mm based on qualitative assessments of raw tracking data files (Gray et al. 2009; Cullen et al. 2010).

Velocity was particularly affected in a phase-related manner by input filter settings (Fig. 1b), and this asymmetry reflected a clear behavioural difference between the two phases. Gregarious locusts tended to make movements of a greater distance than did solitarious locusts, resulting in a significantly higher total distance moved on average (Fig. 2a). By contrast, many of the movements made by solitarious locusts covered much shorter distances, which often included a greater degree of turning on the spot (returning a higher amount of 'turning and small body movements', formerly 'mean angular velocity'; see Fig. 2c and 2e). Since increasing

the MDM filter first affected those individual movements of shorter distances, it also served to disproportionately remove informative displacement data from solitarious tracks above a stringency of 3 mm.

Activity-related variables

Previous logistic regression models of locust phase have retained variables related to overall movement; other work investigating *C. terminifera* retained either distance moved (Gray et al. 2009) or velocity (Cullen et al. 2010). However, some models built for other species included moving time instead of distance moved, either as a raw value in seconds (e.g. Roessingh & Simpson 1994; Despland & Simpson 2000; Sword 2003) or as a fraction of the overall duration of the assay (e.g. McCaffery et al. 1998; Hoste et al. 2002a) (these amount to the same thing when the assay duration is fixed, of course), while others retained movement frequency, the number of individual walking events that a locust made during the assay (e.g. Hägele & Simpson 2000; Simpson et al. 2001). (For a comprehensive list, see Supplementary Table 1).

Whereas the specific activity-based variable/s retained by the final model is/are not necessarily important *per se*, the final list of behaviours included in the model building process should not include variables that considerably overlap. To include distance moved, moving time, movement frequency and mean velocity for consideration by the same model involves a degree of redundancy. We chose only to consider distance moved and mean velocity, as we believe that these alone were sufficient to represent the scale of movement carried out by an individual, over both distance and time, without returning the same behavioural information. Our results

supported this approach. Gregarious locusts returned a total distance moved that was almost 14 times higher than that of their solitarious counterparts, while mean velocity was the only activity-related variable that changed significantly over time. This was likely due to a heightened state of arousal upon first entering the behavioural arena but, nevertheless, locusts remained clearly distinguishable by phase throughout the assay duration.

Furthermore, the link between distance moved and mean velocity exhibited a phase-related difference (Fig. 2d). Normal scores for these variables correlated positively for gregarious insects, demonstrating the expected outcome that faster animals tend to cover more ground over a fixed duration of time. By contrast, the solitarious locusts showed a weaker correlation resulting from the presence of two distinct behavioural subsets. Total distance moved and mean velocity correlated positively for one cohort, following a similar trend to the gregarious phase animals, while a second cluster of outlying points contained locusts with a high mean velocity but a low total distance moved. These individuals exhibited the classic solitarious trait in which, upon entry to the behavioural arena, they quickly moved to the wall opposite the stimulus group before remaining motionless for the rest of the assay. This elegantly demonstrates the capability of an automated tracking system to distinguish specific, pertinent behavioural traits, via the combined consideration of multiple unrefined measurements.

Position-related variables

Attraction to, or repulsion from, a group of conspecifics is among the most strongly defining characteristics of locust behavioural phase state (Simpson et al.

1999; Pener & Simpson 2009). Previous behavioural assays have typically divided the behavioural arena into five zones; (i) stimulus wall (behind which sits the stimulus group of conspecifics), (ii) third of the arena nearest to the stimulus group (stimulus third), (iii) centre third, (iv) third of the arena furthest from the stimulus group (non-stimulus third), and (v) non-stimulus wall. The total time spent in each zone is then returned for each individual locust trial (e.g. Roessingh et al. 1993; Anstey et al. 2009; Cullen et al. 2010). The usefulness of this approach for *C. terminifera* was not entirely supported by the data shown in Fig. 3 and Supplemental Fig. 1.

None of the 123 solitarious locusts assayed spent any amount of time climbing the stimulus wall. While this clearly demonstrates the importance of responses to the position of conspecifics as an attribute of locust behavioural phase, this measurement alone is not a universally informative descriptor because only 54.7% of the gregarious locusts actually climbed on the stimulus wall. Indeed, gregarious insects spent almost as much time at the non-stimulus end of the arena as they did immediately adjacent to the group of conspecifics (Supplementary Fig. 1). This was at least in part due to the size of the arena, since the greater total distance moved by gregarious locusts led to their movement around the entire perimeter of the behavioural arena, and did not limit them to the region closest to the stimulus group. A similar effect was noted in the original assays of *S. gregaria* by Roessingh et al. (1993, see Fig. 4 therein).

It remains an open question whether attraction of gregarious locusts to the stimulus group is masked by the small size of the arena, or is a reflection of attraction being a weaker force in gregarious insects than is repulsion among solitarious insects. Ellis (1959, 1963a; Ellis & Pearce 1962) showed that gregarious *L. migratoria* and *S. gregaria* nymphs exhibited significantly higher grouping in a circular arena than would be predicted by chance alone, and considered gregariousness to involve

habituation to the presence of others. Geva et al. (2010) later noted a similar response, and extended the habituation hypothesis in advocating the behavioural phase phenomenon as a model for learning and memory (see also Anstey et al. 2009; Ott et al. 2012). Heifetz et al. (1994, 1996) demonstrated a strong difference in aggregation propensity between the two phases within a circular arena, but their 'association index' did not disambiguate solitarious-driven repulsion from gregarious-driven attraction. More recently, models of marching bands of gregarious nymphs indicated a stronger influence of mutual alignment (moving in parallel with others) than of strong attraction on collective behaviour in gregarious nymphs of *S. gregaria* (Buhl et al. 2006). This was further supported by empirical observations in *C. terminifera*, in which band formation in wild populations was shown to result from local alignment interactions over a scale of 13.5 cm or less (Buhl et al. 2011).

Studies of collective behaviour in diverse animal species (see Couzin et al. 2002; Sumpter 2006; Herbert-Read et al. 2011) have identified three concentric 'zones' around an individual animal within a group: an outer zone of attraction, in which an animal will be drawn to conspecifics; an alignment/interaction/orientation zone, in which the individual will align with its moving neighbours (as above for *C. terminifera* in Buhl et al. 2011), and a core zone in which an animal will be repelled by conspecifics. There is no doubt that the zone of repulsion is larger in solitarious nymphs than in their gregarious counterparts, however the Roessingh et al. (1993) assay does not allow attraction and alignment zones to be distinguished because the arena does not facilitate marching.

Climbing

Climbing has previously been used as a significant predictor of phase for all four acridid species investigated with the Roessingh et al. (1993) assay system, and has been retained in many independent behavioural models (e.g. Despland & Simpson 2000; Sword 2003; Chapuis et al. 2008; Gray et al. 2009). Climbing could be considered both an activity and position-related behaviour, which is imposed by the spatial limitations of the assay system; a locust can only climb when it reaches a wall of the arena. 'Edge effects' of this nature are well documented in similar behavioural analyses where, upon reaching a wall or obstacle, insects generally move along it rather than head back into the centre of the arena (Gillett 1973; Deneubourg et al. 1990).

In the present study, 'total climbing time' was shown to be a redundant measurement of time spent on the end walls of the arena (the only two which were backlit). Similarly, 'climbing frequency' was found to be a superfluous measurement of 'total distance moved' in gregarious locusts, and of 'turning and small body movements' for the solitarious phase. By contrast, 'mean climb duration' contributed an interesting phase-related difference that the previous two climbing variables failed to represent, and was also unaccounted for by the retained suite of activity- and position-related variables. Solitarious insects exhibited a higher mean duration of climbing than did gregarious nymphs, despite the fact that the gregarious insects spent more time climbing overall (Fig. 4b). The heightened activity of gregarious insects led to them covering all areas of the arena, during which they continuously moved on and off the wall, whereas solitarious insects tended to reach an arena edge (typically the non-stimulus wall) later than the gregarious cohort but, once they did, they remained there until the end of the trial with little or no further movement (see Fig. 3).

Building an optimized logistic regression model of locust behaviour phase

The logistic regression model derived using values from the subset of refined variables over 8-min proved to be both the most powerful and robust of the five behavioural models constructed. All other models were sub-standard in at least one respect. The 8-min, refined subset returned a model that correctly classified 92.4% of locusts with a non-significant value for the Hosmer-Lemeshow goodness-of-fit test ($P = 0.945$, d.f. = 8), indicating that the model prediction was well calibrated with respect to sub-groups of the model populations (Hosmer & Lemeshow 2000; Table 2a). Of equal importance, however, were the three variables retained in the model: total distance moved, turning and small body movements, and total time spent in the non-stimulus third of the arena. Variable optimization prior to logistic regression led not only to a behavioural model that was both powerful and robust, but it also encapsulated two fundamentally different measures of activity and one measure of position relative to the stimulus group. Although subtle differences between insect cohorts and arenas will inevitably lead to the retention of different specific variables across separate logistic regression behavioural models, an important feature for locust phase-related studies is that they do not exclusively contain activity-related variables. Given that locust behavioural phase state is a multivariate syndrome encompassing both activity- and position-related behaviours, activity alone is not a sufficient measurement because it can be influenced by various factors unrelated to phase, including nutritional state, temperature, and infection (Raubenheimer & Gade 1996; Miller et al. 2009; Hinks & Ewen 1986). It is therefore important that phase-related behavioural models also retain at least one position-related variable, to account for the

individual's behaviour with respect to the stimulus group of conspecifics (Pener & Simpson 2009).

A properly parameterized and calibrated logistic regression behavioural model can then be used to predict the phase state of an experimental locust based on the relative weighted contributions of the retained activity- and position-related variables. As indicated by the Wald significance values (Table 2a), 'total distance moved' was the most significant predictor of phase retained within the model, and the β coefficient indicated a positive effect on *P*(greg). As noted above, any locust that moves less than 105 mm in 8 min will always be classified as closer to a true solitarious insect than a true gregarious one by our model, while animals that move more than 755 mm will always be classified as closer to gregarious. The mean phase-related values for 'total distance moved' reflected this, at 96.6 mm and 1177.6 mm, respectively. Behavioural models of this kind are built with fully gregarious and solitarious insects, but they are subsequently employed in phase-related experiments where locusts are predicted to be transitioning from one phase to another (Simpson et al., 1999). Transitional insects would be expected to move distances that are intermediate to the two extremes, and it is within this range that 'turning and small body movements' and 'non-stimulus third time' exerted a considerable influence on the returned score for *P*(greg)(Fig. 5). The β coefficients for 'turning and small body movements' and 'non-stimulus third time' were negatively weighted, indicating that these variables were higher on average in the solitarious locust subset. The negative β coefficients therefore served to reduce the overall score of *P*(greg) as values for these variables increased. While it is of course important to consider the role of individual behavioural variables in relation to the compound measure, the important benefit of

using a multidimensional representation such as *P*(greg) is that the *interactions* among multiple behavioural variables are considered.

Logistic regression models have been successfully used in multiple studies of locust behavioural phase since Roessingh et al. (1993) (see Supplementary Table 1 for a comprehensive list). While the present study further supports the utility of this method, returning a robust model with 92.4% accuracy, it should be noted that algorithms other than those employed by logistic regression have been used to define multidimensional behavioural states. Frölich et al. (2008) used video analysis to automate the 'forced swimming test' in rodents treated with antidepressants, and showed that support vector machines could be entrained to discriminate between different drug-treated groups with accuracies of up to 90%. Martiskainen et al. (2009) also used support vector machines to recognise discrete behavioural patterns in dairy cattle, albeit with a lower precision of 78%. Future studies could assess the utility of support vector machines in the classification of locust phase, or any other behavioural syndrome, but should still only be applied in conjunction with a variable optimization process as demonstrated here to ensure that an understanding of the underlying behavioural phenotype remains the central goal of the analysis.

CONCLUSIONS

Automated video tracking and logistic regression analysis provides a powerful, efficient, and user-independent tool for assaying locust behavioural phenotypes. In order to produce a biologically meaningful multivariate logistic regression model of locust behavioural phase, data collection via automated video tracking should be complemented by a rigorous variable optimization process. Where

possible, behaviours should only be considered for entry into the logistic regression analysis if they capture some key aspect of the behavioural phenotype. Position-related variables should be examined over a range of time scales to determine the point at which they become truly informative, whilst care should be taken to ensure that climbing-related measurements are not simply edge effects conditional upon other behaviours. Final models should be checked formally (e.g. Hosmer-Lemeshow goodness-of-fit) and informally to assess their biological relevance. We argue that a logistic regression model in which both activity and position-related variables are retained is critical, so as to represent the multi-dimensional nature of locust behavioural phase and avoid potentially confounding effects of several non-phase related factors that can affect locomotion alone.

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FIGURE CAPTIONS

Fig. 1. The effect of increased 'Minimum Distance Moved' input filter (mm) on mean values of four activity-related variables for solitarious locusts (N = 123; solid curve) and gregarious locusts (N = 128; broken curve). Error bars represent 95% CI.

Fig. 2. (a) Graphs displaying the differences in three activity-related variables for solitarious locusts ($N = 123$; solid curve) and gregarious locusts ($N = 128$; broken curve), across each minute of the behavioural assay. Error bars represent 95% CI. (b) Scatterplots of normalized values for 'total distance moved' vs. normalized values for 'mean velocity' for gregarious and solitarious locusts. Solid lines represent best fit. (c) Histograms displaying 'turning and small body movements' (formerly designated 'mean angular velocity') for solitarious and gregarious Australian plague locusts. Arrows indicate mean values.

Fig. 3. Mean time spent in each 1 cm section of behavioural arena by 123 solitarious locusts (black bars) and 128 gregarious locusts (grey bars), across each minute of the assay duration. Section '0' represents the stimulus wall of the arena, while '24' represents the non-stimulus end. Error bars represent 95% CI.

Fig. 4. Mean climb time vs. total climbing time. (a) Scatterplot of normalized values for both variables, (b) bar graph highlighting the differences between solitarious locusts ($N = 123$, black bars) and gregarious locusts ($N = 128$, grey bars). Error bars represent 95% CI.

Fig. 5. The effect of 'turning and small body movements' and 'non-stimulus third time' on the returned value for P(greg). The maximum time spent in the stimulus third (480 s) is represented by the dashed line, while the minimum time (0 s) is represented by the solid line. In both cases, 'total distance moved' is set to 430 mm (explained in text).

TABLE CAPTIONS

Table 1. Behavioural variables recorded by Ethovision, and considered/optimized prior to the model-building process. Activity-related variables are indicated with an asterisk; these were all subject to the 3 mm MDM input filter (see text). 'Refined variables' formed the subset of seven behaviours entered for consideration by the final optimized model (see Table 2a).

Table 2. Behavioural variables, and their respective coefficients, retained by forward step-wise logistic regression analysis of 123 solitarious and 128 gregarious *C. terminifera* final-instar nymphs. Negative coefficients indicate that the corresponding variable was, on average, greater in solitarious locusts than the gregarious cohort. The Wald statistic indicates the significance of the contribution made by each retained variable to the overall model. The Hosmer and Lemeshow goodness-of-fit test was not significant, indicating an acceptable fit. Behavioural model: $\eta=\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_k X_1$, with $P(\text{greg}) = e^{\eta}/(1 + e^{\eta})$.

SUPPLEMENTARY MATERIAL CAPTIONS

Supplementary Table 1. Summary of behavioural models built in previous analyses of locust phase polyphenism. Blanks indicate where data were not available from the cited source.

Supplementary Fig. 1. Position-related variables. (A) Mean time spent in each third of the behavioural assay arena by 123 solitarious locusts (black bars) and 128

gregarious locusts (white bars). (B) Mean distance from stimulus group at the end of the assay (x–distance). Error bars represent 95% CI.

Supplementary Fig. 2. Scatterplots of rank-normalized values for (A) climbing time vs. time spent on the stimulus and non-stimulus walls, and (B) climbing frequency vs. distance moved. Positive correlations indicate that climbing time and climbing frequency are redundant measures of position and activity, respectively.

Figure 1

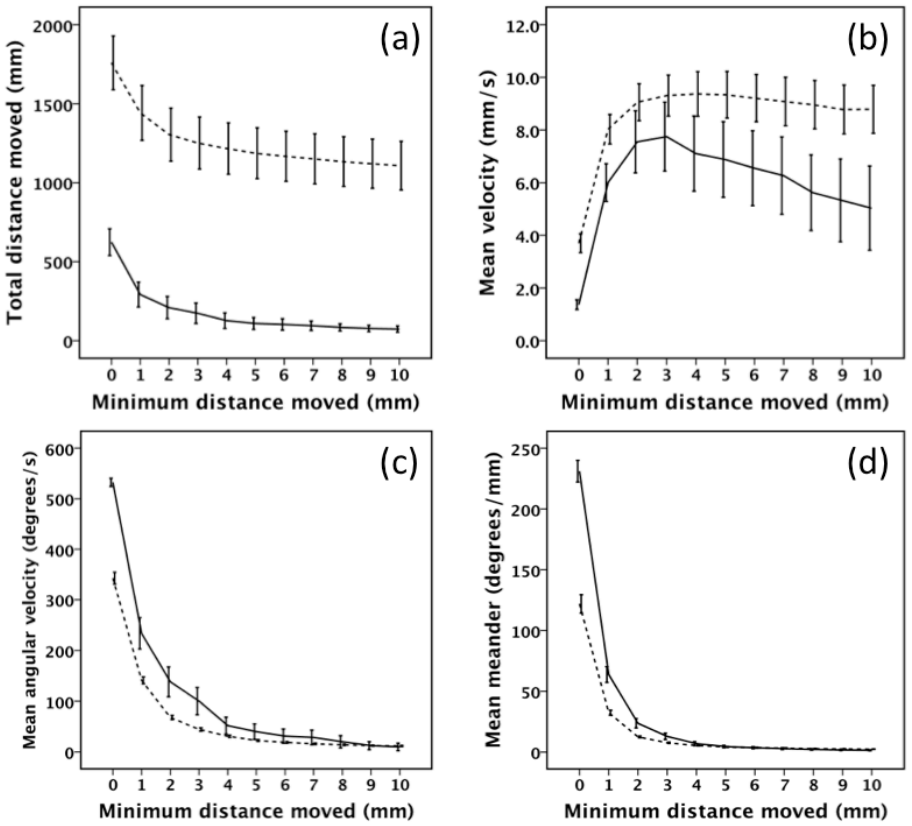

(a)
Total distance moved (mm)
2000
1500
1000
500
0
0 1 2 3 4 5 6 7 8 9 10
Minimum distance moved (mm)
(b)
Mean velocity (mm/s)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0 1 2 3 4 5 6 7 8 9 10
Minimum distance moved (mm)
(c)
Mean angular velocity (degrees/s)
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10
Minimum distance moved (mm)
(d)
Mean meander (degrees/mm)
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10
Minimum distance moved (mm)

Figure 2

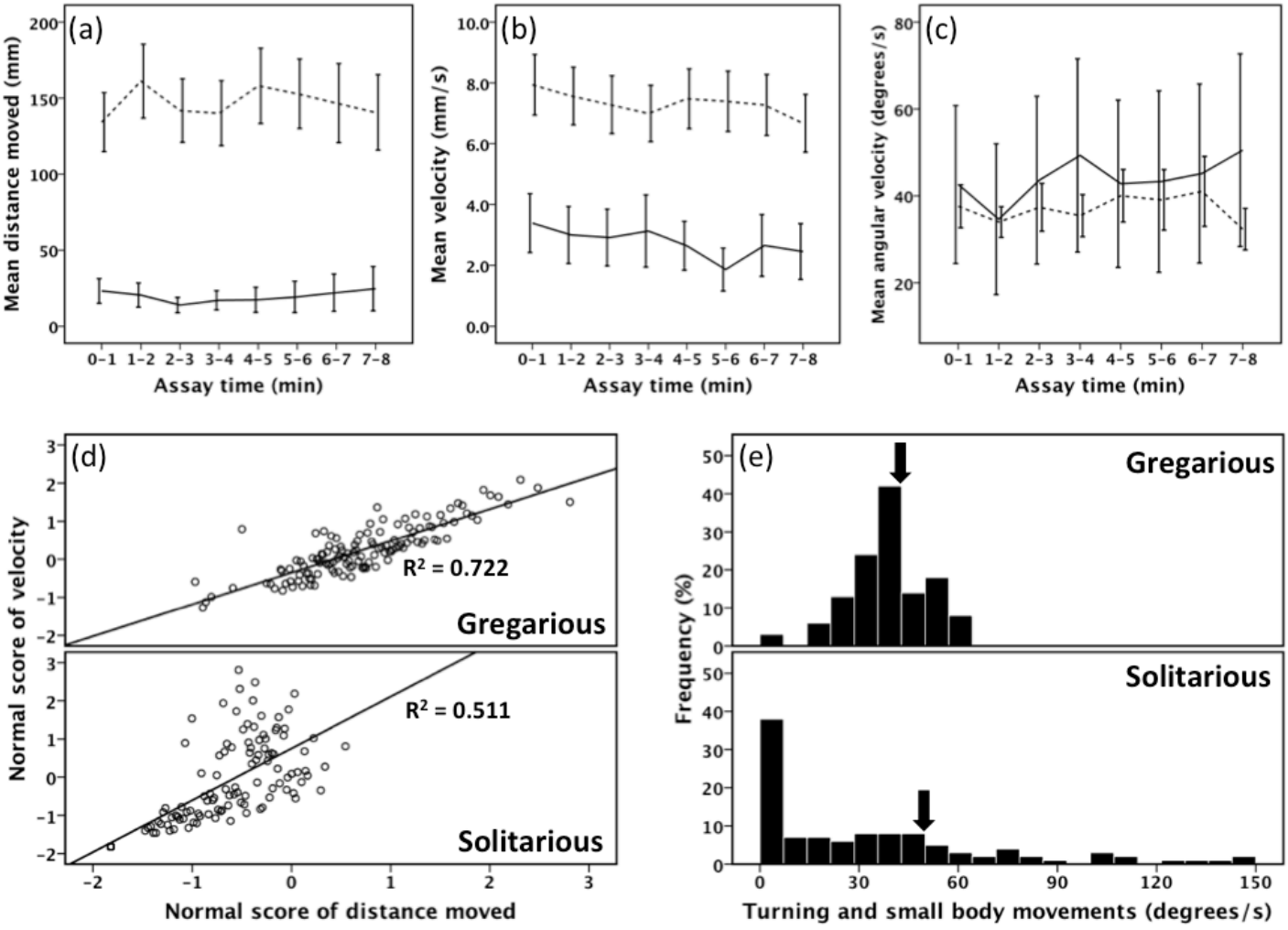

(a)
Mean distance moved (mm)
200
150
100
50
0
0-1 1-2 2-3 3-4 4-5 5-6 6-7 7-8
Assay time (min)
(b)
Mean velocity (mm/s)
10.0
8.0
6.0
4.0
2.0
0.0
0-1 1-2 2-3 3-4 4-5 5-6 6-7 7-8
Assay time (min)
(c)
Mean angular velocity (degrees/s)
80
60
40
20
0-1 1-2 2-3 3-4 4-5 5-6 6-7 7-8
Assay time (min)
(d)
Normal score of velocity
R² = 0.722
Gregarious
R² = 0.511
Solitarious
Normal score of distance moved
(e)
Gregarious
Solitarious
Frequency (%)
Turning and small body movements (degrees/s)
0
30
60
90
120
150

Figure 3

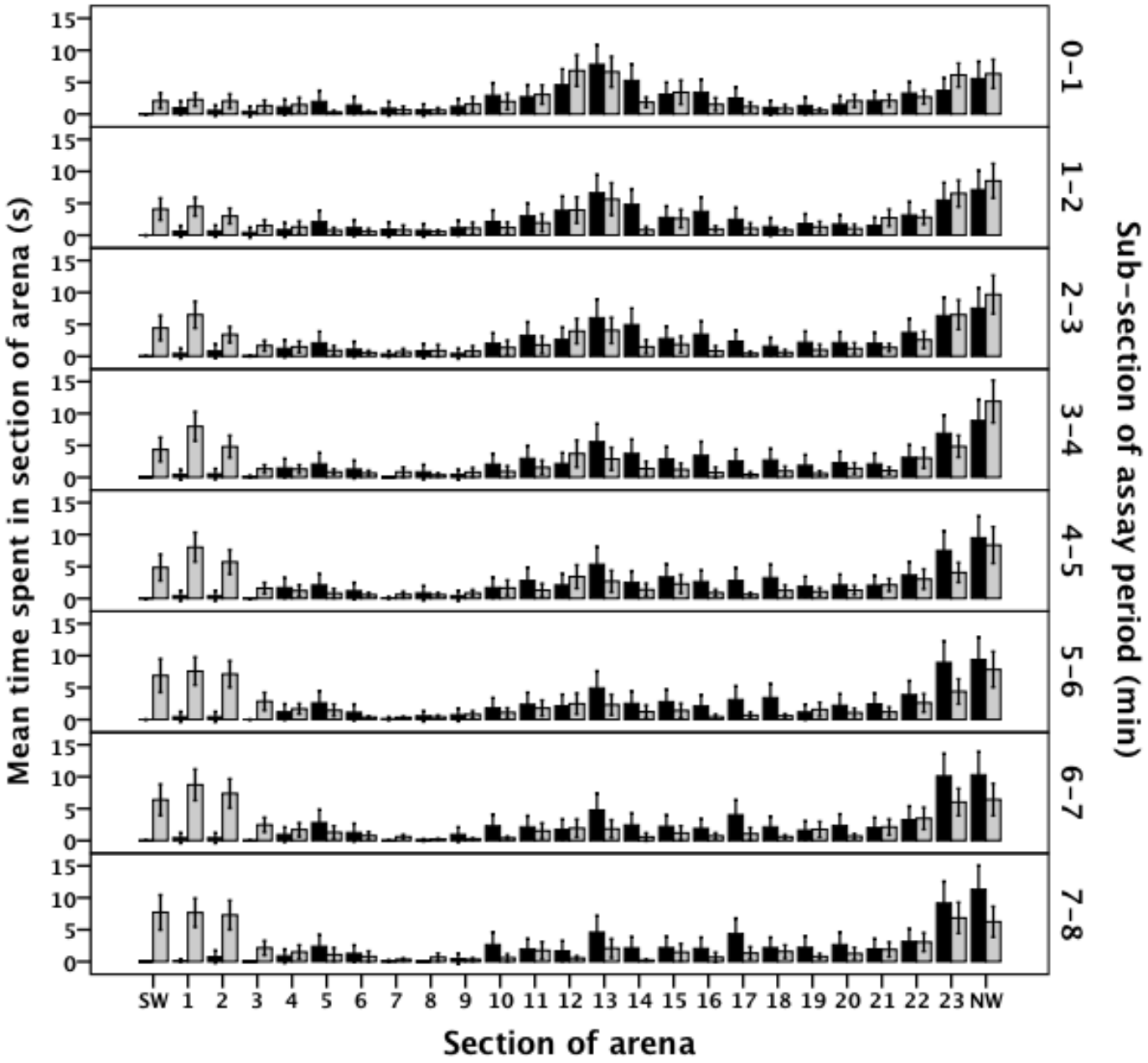

Mean time spent in section of arena (s)
15
10
5
0
0–1
1–2
2–3
3–4
4–5
5–6
6–7
7–8
Sub-section of assay period (min)
SW 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 NW
Section of arena

Figure 4

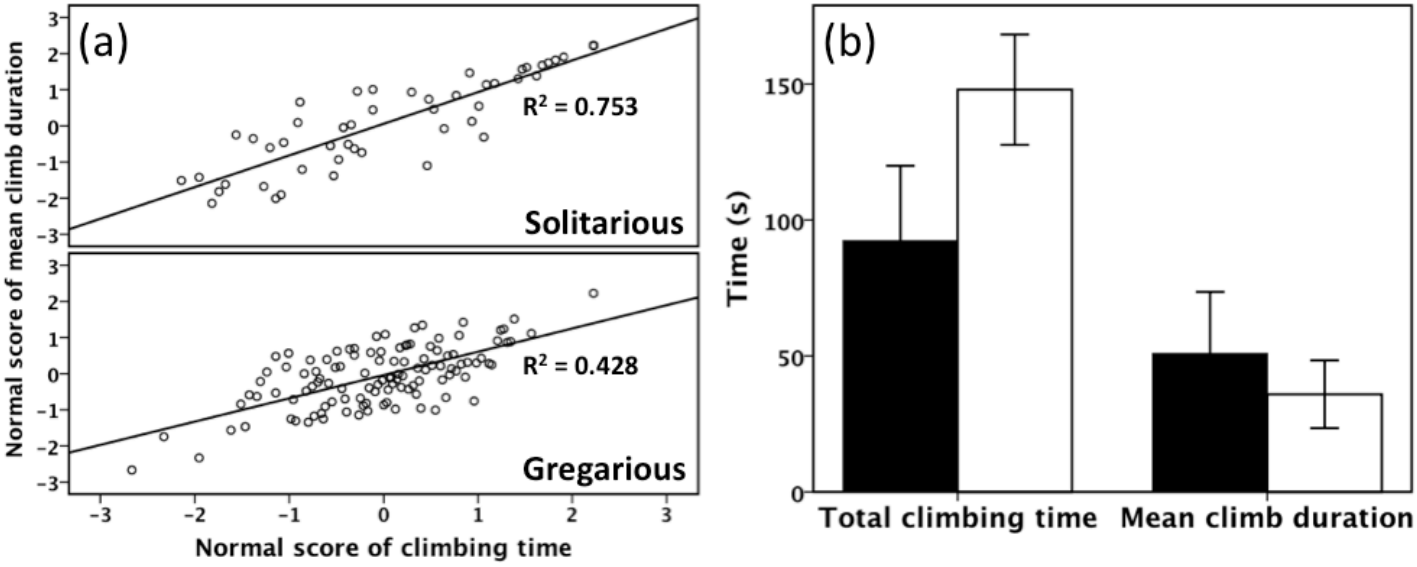
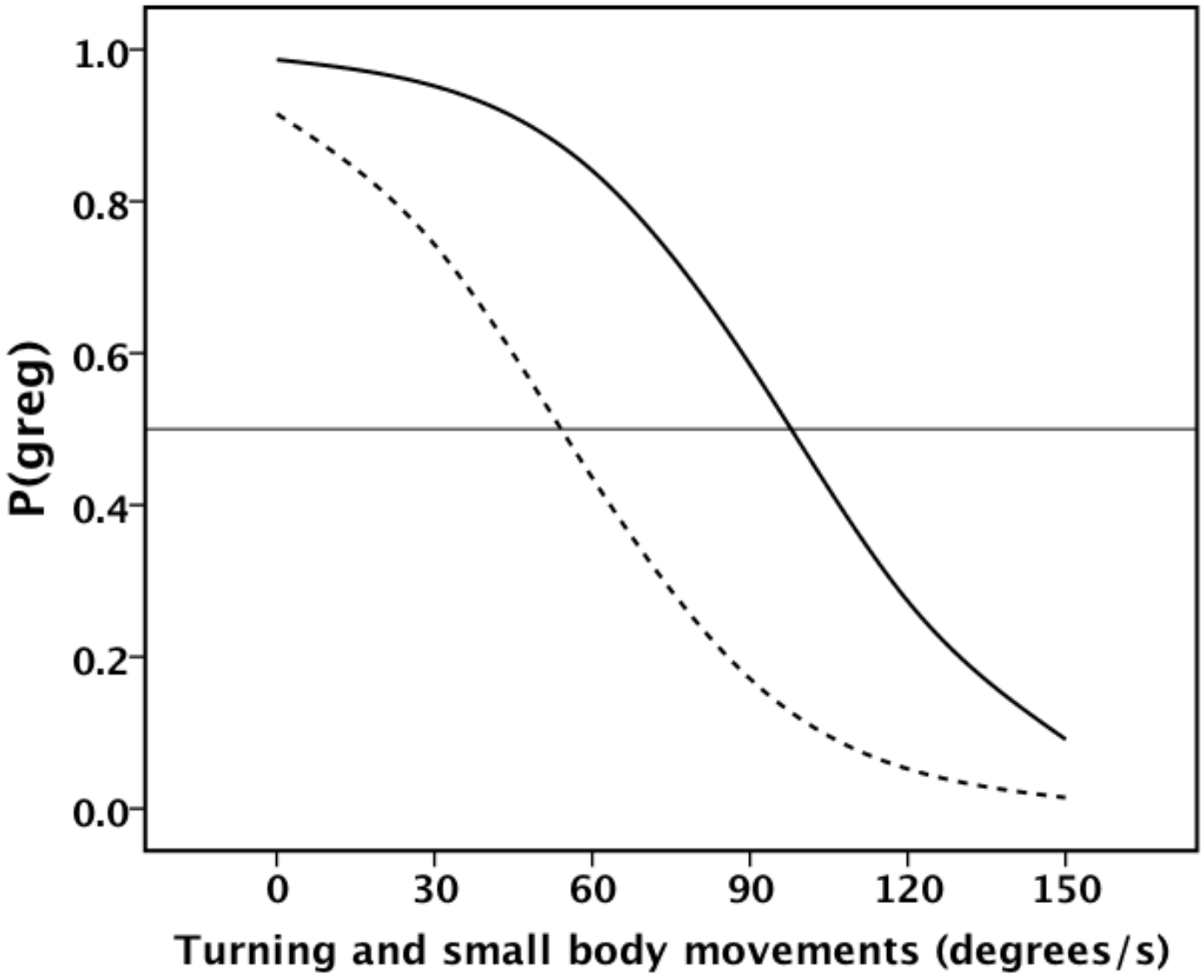
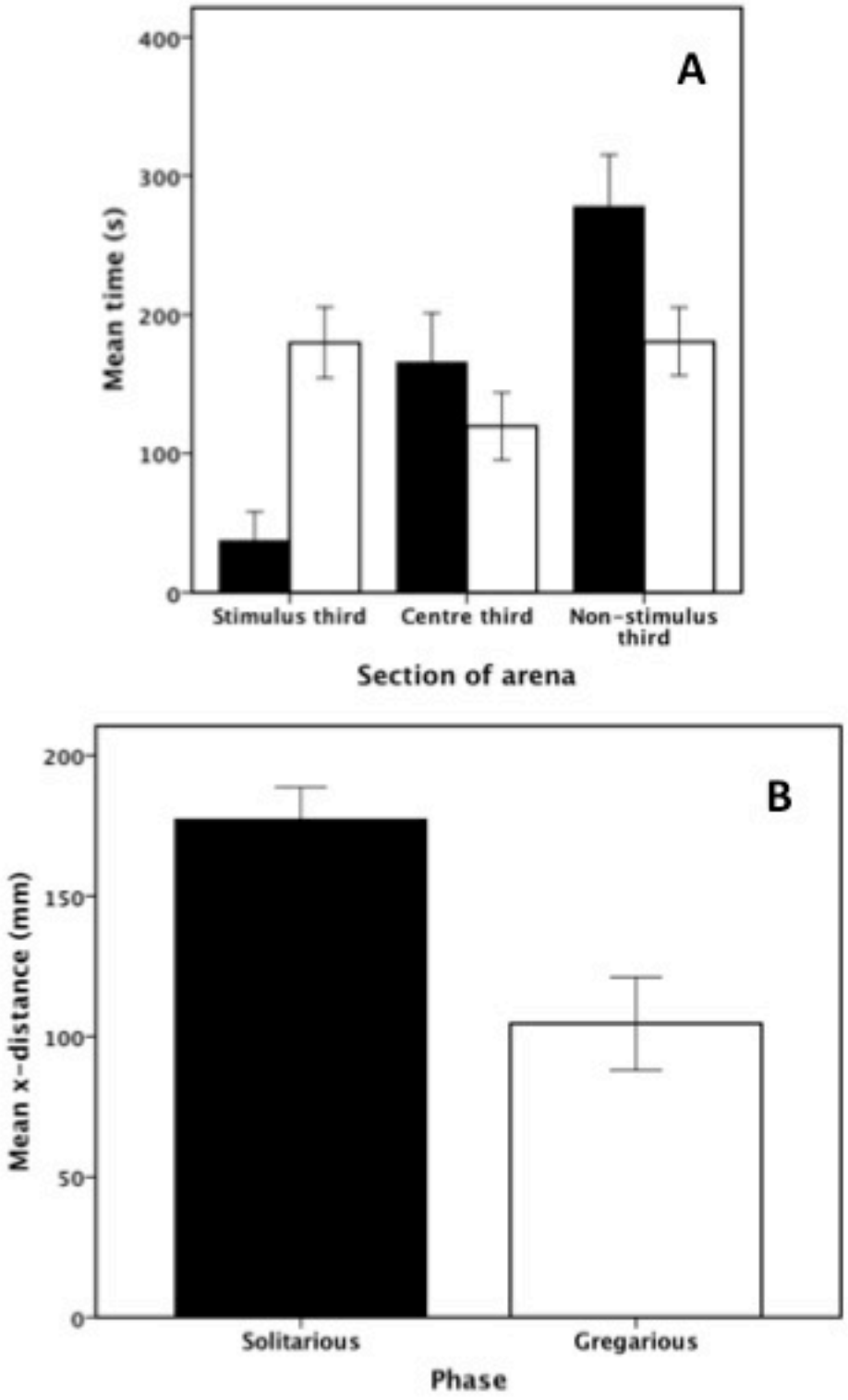

(a)
R² = 0.753
Solitarious
R² = 0.428
Gregarious
Normal score of mean climb duration
Normal score of climbing time
(b)
Time (s)
150
100
50
0
Total climbing time
Mean climb duration

Figure 5


1.0
0.8
0.6
0.4
0.2
0.0
P(greg)
0
30
60
90
120
150
Turning and small body movements (degrees/s)

Supplementary Fig. 1



Supplementary Fig. 2

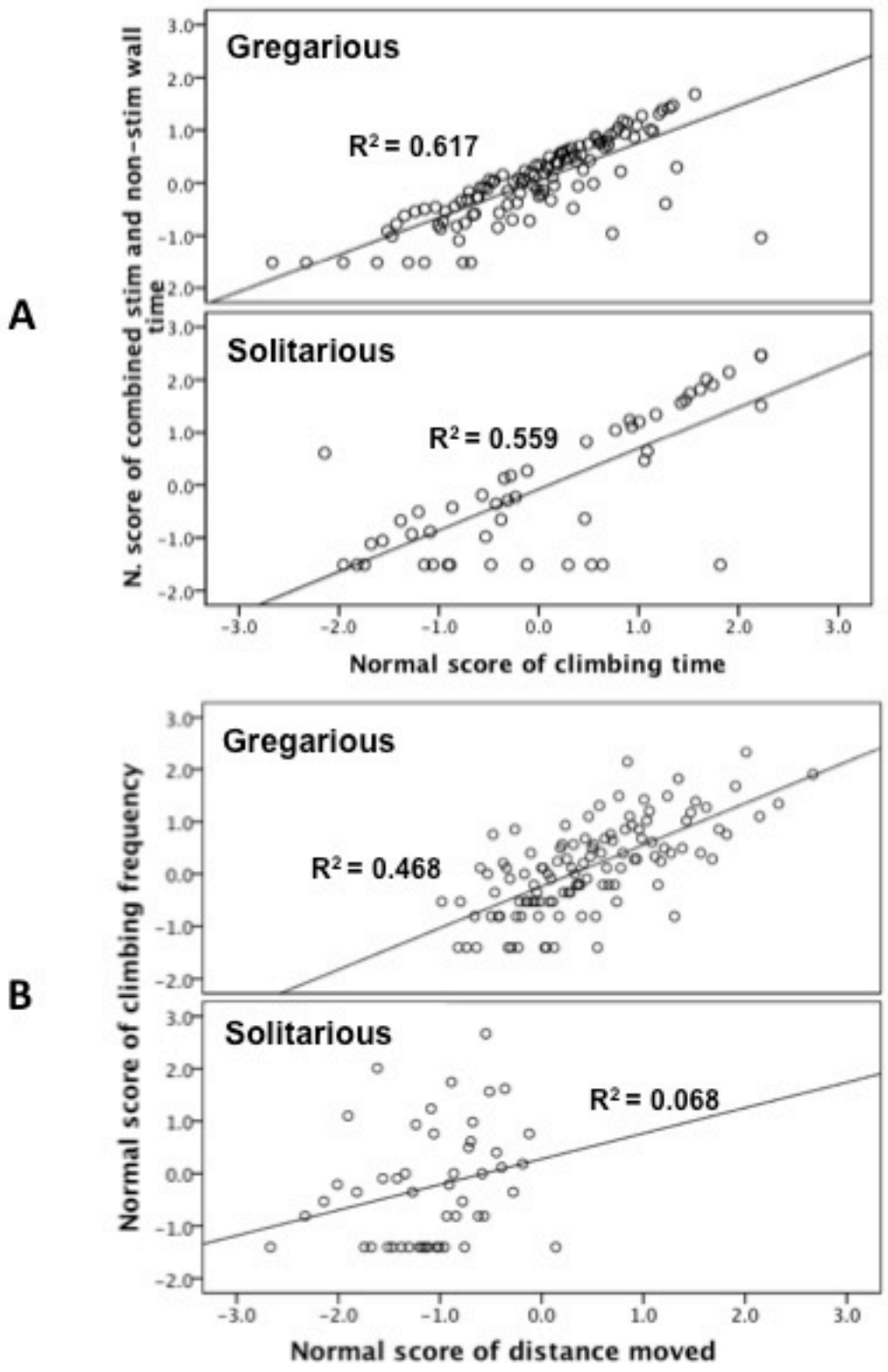


Table 1

Total variable list	Definition/comment
Total distance moved (mm)*	Distance moved across the entire assay duration
Mean velocity (mm s–1)*	Calculated as the total distance moved (mm) divided by the total time spent moving (s)
Mean angular velocity (degrees s–1)*	A measure of turning, renamed below
Mean absolute meander (degrees mm–1)*	A measure of path tortuosity
Stimulus wall time (s)	Total time climbing the stimulus wall
Stimulus third time (s)	Not including time spent on the stimulus wall
Centre third time (s)	Time spent in the central third of the arena
Non-stimulus third time (s)	Not including time spent on the non-stimulus wall
Non-stimulus wall time (s)	Total time climbing the non-stimulus wall
X – distance (mm)	The insects' distance from the stimulus wall at the end of the assay
Total climbing time (s)	Total time spent climbing all four walls of the arena
Climbing frequency (count)	The number of individual wall mounting events
Mean climb duration (s)	The mean duration of each individual wall mounting event
Refined variable list	**Definition/comment**
Total distance moved (mm)*	Distance moved across the entire assay duration
Mean velocity (mm s–1)*	Calculated as the total distance moved (mm) divided by the total time spent moving (s)
Turning and small body movements (degrees s–1)*	Mean absolute angular velocity, renamed
Stimulus third time (s)	Re-defined as the total time spent in the stimulus third of the arena and climbing the stimulus wall
Non-stimulus third time (s)	Re-defined as the total time spent in the non-stimulus third of the arena and climbing the non-stimulus wall
X – distance (mm)	The insects' distance from the stimulus wall at the end of the assay
Mean climb duration (s)	The mean duration of each individual wall mounting event

a. 8 min. model, selected variables				
Retained variable	**Coefficient, β**	**β, s. e.**	**Wald statistic**	**Sig.**
Distance moved	0.013	0.002	31.074	0.000
Turning and small body movements	–0.044	0.017	7.071	0.008
Time in non-stimulus third	–0.004	0.002	5.649	0.017
Constant	–1.288	0.388	11.032	0.001

(96.7% solitarious correct, 88.3% gregarious correct, **92.4% overall.** Hosmer-Lemeshow goodness-of-fit test, $P = 0.945$, d.f. = 8)

b. 6 min. model, selected variables				
Retained variable	**Coefficient, β**	**β, s. e.**	**Wald statistic**	**Sig.**
Distance moved	0.005	0.001	42.145	0.000
Turning and small body movements	–0.012	0.006	4.498	0.034
X – distance	–0.005	0.002	5.572	0.018
Constant	–0.263	0.408	0.519	0.769

(95.1% solitarious correct, 72.7% gregarious correct, **83.7% overall.** Hosmer-Lemeshow goodness-of-fit test, $P = 0.000$, d.f. = 8)

c. 4 min. model, selected variables				
Retained variable	**Coefficient, β**	**β, s. e.**	**Wald statistic**	**Sig.**
Distance moved	0.007	0.001	45.43	0.000
Velocity	–0.100	0.04	6.24	0.012
X – distance	–0.005	0.002	4.089	0.043
Constant	–0.224	0.409	0.298	0.585

(92.7% solitarious correct, 72.7% gregarious correct, **82.5% overall.** Hosmer-Lemeshow goodness-of-fit test, $P = 0.010$, d.f. = 8)

d. 2 min. model, selected variables				
Retained variable	**Coefficient, β**	**β, s. e.**	**Wald statistic**	**Sig.**
Distance moved	0.011	0.002	38.265	0.000
Velocity	–0.115	0.041	7.837	0.005
Constant	–0.716	0.209	11.694	0.001

(91.9% solitarious correct, 65.6% gregarious correct, **78.5% overall.** Hosmer-Lemeshow goodness-of-fit test, $P = 0.002$, d.f. = 8)

e. 8 min. model, unrefined variables				
Retained variable	**Coefficient, β**	**β, s. e.**	**Wald statistic**	**Sig.**
Distance moved	0.012	0.002	37.899	0.000
Meander	0.096	0.04	5.738	0.017
Turning and small body movements	–0.050	0.009	28.971	0.000
Time in centre third	0.003	0.001	4.508	0.034
Constant	–3.052	0.627	23.715	0.000

(95.1% solitarious correct, 88.5% gregarious correct, **91.7% overall.** Hosmer-Lemeshow goodness-of-fit test, $P = 0.030$, d.f. = 8)

Supplementary Table 1. Summary of behavioural models built in previous analyses of locust phase polyphenism.

Locust species	Life stage	Assay type	Assay duration (s)	Logistic regression model	Variables retained by the logistic regression model		Model accuracy, where given	Reference(s)
					Related to activity	Related to position w.r.t. stimulus group of conspecifics		
Schistocerca gregaria	Hatchlings	Manual	Variable, up to 400	Backwards, stepwise	Track straightness, velocity, angle/turn, distance moved, grooming frequency, jumping frequency, walking frequency, walking time fraction	None	92.1%	Islam et al., 1994a
	Hatchlings	Manual	Variable, up to 400	Backwards, stepwise	Velocity, distance moved, grooming frequency, jumping frequency, leg movement frequency	x-distance	87.7%	Islam et al., 1994b
	Hatchlings	Manual	600	Forward, stepwise	climbing frequency, walking frequency, climbing time fraction, grooming time fraction	x-distance	83.9%	Bouaïchi et al., 1995
	Hatchlings	Manual	Variable, up to 400	-	Track straightness, velocity, angle/turn, distance moved, grooming frequency, jumping frequency, walking frequency, walking time fraction, crouch frequency, leg movement frequency	x-distance	-	McCaffery et al., 1998
	Hatchlings	Manual	-	-	velocity, turning frequency, resting time fraction, walking frequency, distance moved, climbing time fraction	None	-	Hägele et al., 2000
	2nd instar nymphs	Manual	300	Forward, stepwise	Time climbing, time grooming, time resting, velocity, walking time, short movements**	x-distance	93.0%	Despland and Simpson, 2000
	2nd instar nymphs	Manual	300	Forward, stepwise	Climbing time fraction, grooming time fraction, short movements**, resting time fraction, velocity, moving time fraction	x-distance	93.3%	Despland et al., 2000
	Final instar nymphs, second instar nymphs	Manual	Variable, up to 600	Forward, stepwise	Jump frequency, leg movement frequency, reposition body frequency, grooming frequency, walking time	x-distance	89.8%	Roessingh et al., 1993; Collett et al., 1998; Lester et al., 2005
	Final instar nymphs	Manual	-	-	Climbing time fraction, grooming frequency, walking frequency	x-distance	97.5%	Roessingh et al., 1998
	Final instar nymphs	Manual	600	Forward, stepwise	Track straightness, grooming frequency, crouching time, walking time	x-distance	-	Roessingh and Simpson, 1994
	Final instar nymphs	Manual	500	-	Velocity, resting time fraction, walking frequency, grooming time fraction	x-distance	93.3%	Hägele and Simpson, 2000
	Final instar nymphs	Manual	300	Backwards, stepwise	Movement frequency, velocity, grooming time fraction	Stimulus third fraction	88.4%	Sword and Simpson, 2000
	Final instar nymphs	Manual	500	-	Walking frequency, grooming frequency, resting time	Uncertain- stimulus third time or x-distance	92.2%	Simpson et al., 2001; Rogers et al., 2003
	Final instar nymphs	Manual	300	Forward, stepwise	Touching antenna time, grooming time, small leg movements, swaying time, walking time fraction	Stimulus third time	83.3%	Hoste et al., 2002a
	Final instar nymphs	Manual	500	Forward, stepwise	Walking speed, resting time, grooming time	Stimulus third time	90.0%	Anstey et al., 2009; Ott et al., 2012
	Adults	Manual	-	-	Climbing time fraction, grooming frequency, walking frequency	None	96.8%	Roessingh et al., 1998
	Adults	Manual	600	Forward, stepwise	Climbing time fraction, grooming frequency, walking, head movement	x-distance	90.6%	Bouaïchi et al., 1995
Schistocerca americana	Final instar nymphs	Manual	300	No model built	Climbing time, turn angle, turn frequency, walk frequency, velocity, moving time	x-distance, overall position*	-	Sword, 2003
Locusta migratoria	Hatchlings	Manual	180	Box-Cox transformation, not logistic regression	Track straightness, velocity, angle, turning rate, duration of movement, jumping frequency, climbing time, body-swaying frequency	End coordinate, stimulus third time, near-lit-wall time	-	Chapuis et al., 2008
	Final instar nymphs	Manual	180	Forward, stepwise	Turn rate, single leg movement count	Non-stimulus third time	70.2%	Hoste et al., 2002b
	Fourth instar nymphs	Automated (Ethovision)	300	Forward, stepwise	Distance moved, moving time	Attraction index*	87.1%	Guo et al., 2011
	Final instar nymphs	Automated (Ethovision)	300	Forward, stepwise	Moving time	Attraction index*	-	Ma et al., 2011
Chortoicetes terminifera	Final instar nymphs	Automated (Ethovision)	480 - 600	Forward, stepwise	Distance moved, climbing time	Mean distance to stimulus group	98.7%	Gray et al., 2009
	Final instar nymphs	Automated (Ethovision)	480	Backwards, stepwise	Mean velocity, mean angular velocity	Time spent on non-stimulus wall, time spent in non-stimulus third of arena	94.7%	Cullen et al., 2010
	Final instar nymphs	Automated (Ethovision)	480	Forward, stepwise	Distance moved, turning and small movements (mean angular velocity)	Time spent in the non-stimulus third of the arena	92.4%	This study.

* 'overall position' and 'attraction index' are indices that weigh the difference in time spent at both ends of the arena

** 'short movements', defined as a move forwards of less than one body length per unit time

Blanks indicate where data were not available from the cited source.